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To aid in the assessment of the risk of Air Force personnel working with hydrocarbon fuels and compounds, an attempt was made to further characterize the nephropathy that results from exposure to hydrocarbons. The purpose of this study was to isolate and establish purified primary cultures of male rat proximal tubular cells suitable for experimental exposure to sublethal concentrations of solubilized 2,3, 4-trimethylpentane (TMP), a model hydrocarbon. Experiments were conducted to evaluated the cytotoxicity and metabolism of solubilized TMP in media containing or lacking the protein albumin.

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THE EFFECT OF 2,3,4-TRIMETHYLPENTANE ON THE ULTRASTRUCTURE OF PROXIMAL TUBULAR CELLS IN PRIMARY CELL CULTURE

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To aid in the assessment of the risk of Air Force personnel working with hydrocarbon fuels and compounds, an attempt was made to further characterize the nephropathy that results from exposure to hydrocarbons. The purpose of this study was to isolate and establish purified primary cultures of male rat proximal tubular cells suitable for experimental exposure to sublethal concentrations of solubilized 2,3,4-trimethylpentane (TMP), a model hydrocarbon. Experiments were conducted to evaluate the cytotoxicity and metabolism of solubilized TMP in media containing or lacking the protein albumin.

Proximal tubule cells in primary suspension culture were exposed to one of the following levels of TMP: 7.9, 12.0, 15.7, 19.1 or 25.5 mM. After 4 hours of exposure, pelleted cells were fixed for transmission electron microscopy by resuspension in 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1M cacodylate buffer at pH 7.4. After a minimum fixation of at least 24 hours, the cells were post-fixed with 2% osmium tetroxide in 0.1M cacodylate buffer at pH 7.4. Cells were processed into Polybed 812 plastic capsules. Sections one micron thick were cut in order to verify that cells were intact and suitable for thin sectioning. Thin sections (60-90 nm) were cut on an ultramicrotome using a diamond knife. Thin sections, stained with uranyl acetate and lead citrate, were examined with a transmission electron microscope at 60 kV. Photographs of representative proximal tubule cells were taken at three levels of magnification.

All control proximal tubular (PT) cells contained vacuoles to varying degrees with a minimal degree of mitochondrial swelling. The outer compartment of many mitochondria appeared to be slightly swollen as evidenced by intracristal swelling. There were no significant differences between controls with albumin and controls without albumin (FIG 1 and 2). As PT cells were exposed to higher doses of TMP, the number of viable and intact cells decreased. Cell viability was not quantitated at the ultrastructural level. Albumin in the media did not confer a protective effect on PT cells as previously seen with hepatocytes in culture.[1] However, albumin did allow slightly greater changes to occur in rough endoplasmic reticulum and mitochondria (FIG 3). No apparent differences were seen between albumin and non-albumin groups for nuclei, lipid, smooth endoplasmic reticulum, vacuoles, and microvilli (FIG 4).

Primary cultures of rat kidney proximal tubular cells can be exposed to a chemical such as TMP. Albumin in the media does not appear to confer a protective effect as seen with hepatocytes in primary culture. Media was analyzed for the presence of TMP metabolites. Although metabolites had previously been isolated in urine of dosed rats[2] and more recently in the culture media of primary hepatocytes exposed to TMP[3], no metabolites were detected from the kidney cells. This confirms that primary metabolism of TMP occurs in the liver, not in the kidney, even though the kidney is the target organ for toxicity.

1. Del Raso, N.J. and Mattie D.M. The Toxicolgist 8(1):217, 1988
2. Olson, C.T., Hobson, D.W., Yu, K.O. and Serve, M.P. Toxicology Letters 37:199-202, 1987
3. Del Raso, N.J. Personal communication, 1989
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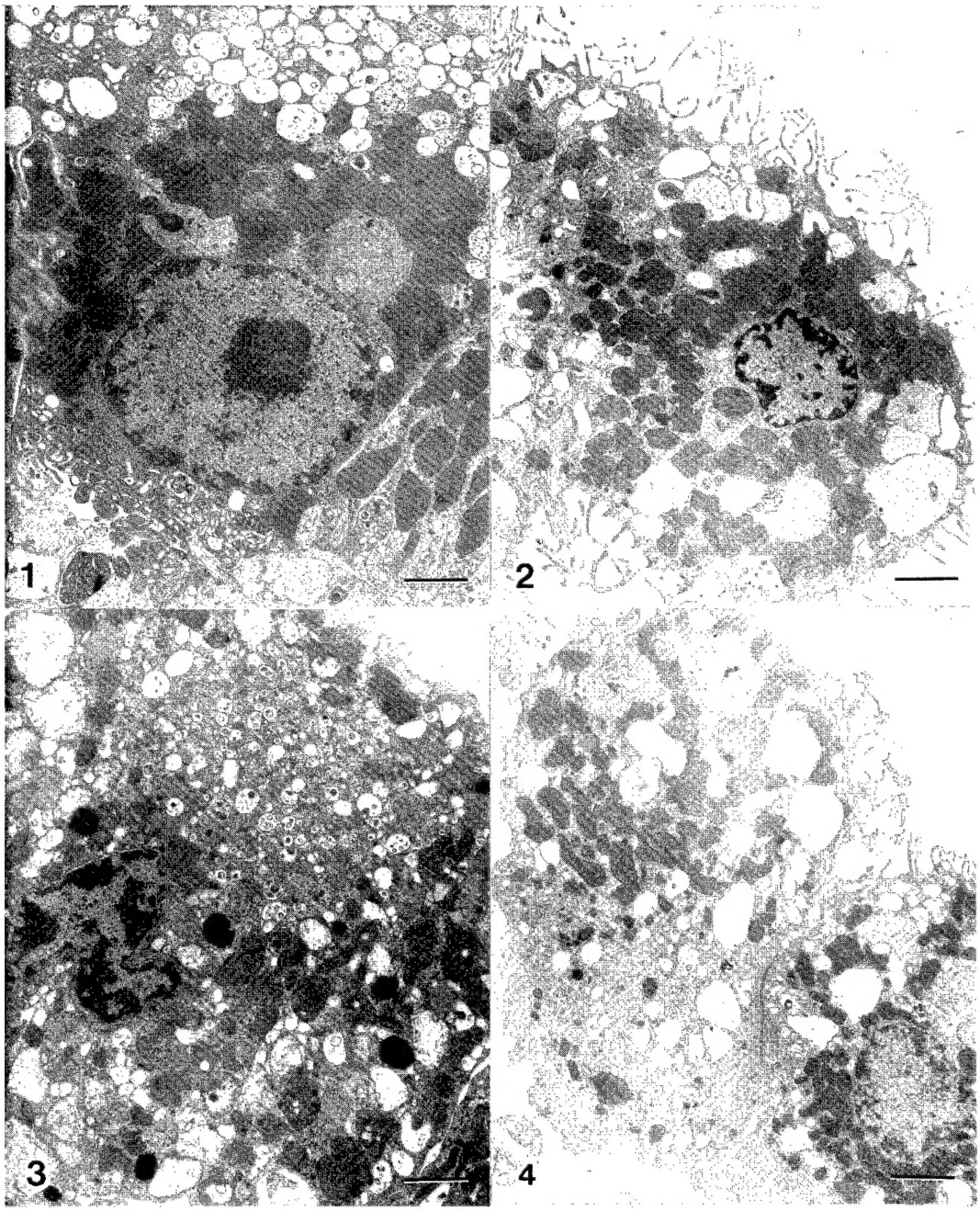

FIG. 1.--Control proximal tubule cell with albumin. Bar = 1 um.
FIG. 2.--Acetone control proximal tubule cell without albumin. Bar = 1 um.
FIG. 3.--Proximal tubule cell with albumin exposed to 12.0 mM TMP for 4 hours. Bar = 1 um.
FIG. 4.--Proximal tubule cell without albumin exposed to 7.9 mM TMP for 4 hours. Bar = 1 um.